

## Study of Ascitic Fluid in Children with Chronic Liver Disease in Different Variants of Peritonitis at a Tertiary Care Hospital, Bangladesh

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### Abstract

### Original Research Article

**Background:** Chronic liver disease (CLD) is not uncommon in Bangladesh. Ascites is common feature of CLD patients. Ascites is a culture media for bacterial infection. Spontaneous bacterial peritonitis is a frequent complication of ascites in children with chronic liver disease. The rapid and effective diagnosis of peritonitis will reduce mortality.

**Aim:** The aim of this study is to see the variants of ascitic fluid infection in children with chronic liver disease.

**Methods:** It is a cross sectional observational study. This study was conducted at the department of Pediatric Gastroenterology and Nutrition, BSMMU, Dhaka, Bangladesh without interrupting standard care practiced in the department. The study was done over a period of one & half year, from January 2016 through July 2017. During this period consecutive children CLD with ascites were included in this study. Sample was collected purposively who was fulfilling inclusion criteria. The details history, physical examination findings and investigation reports were recorded in a predesigned standard data sheet. History was obtained directly from the parents, which include jaundice, abdominal pain, fever, diarrhea, family history of liver diseases or other relevant medical histories. Investigations were done for diagnosis of chronic liver disease & identify the cause. Ascitic fluid study especially physical appearance, cytology, total protein, LDH, Gram stain & culture were done in all case. Statistical analysis was done using Statistical Package for Social Science 20.0 (SPSS; Chicago, Illinois) for Windows XP. **Results:** A total of 30 children were selected according to selection criteria. After ascitic fluid study, all patients were divided into two groups: Group I included five patients (16.67%) with culture negative neutrocytic ascites (CNNA) in which the neutrophil count  $\geq 250/\text{mm}^3$  and culture was negative indicate infected group. Group II, twenty five (83.33%) patients in which the neutrophil count  $< 250/\text{mm}^3$  and negative culture indicate non infected group. None of our patients had spontaneous bacterial peritonitis (SBP) or bacterascites. Presence of fever, history of abdominal pain and tenderness significantly higher in CNNA group ( $p < 0.05$ ). **Conclusions:** Culture negative neutrocytic ascites (CNNA) was the only variety of ascitic fluid infection in this study. Infected cases may be asymptomatic. Clinical features of ascitic fluid infection are needed to differentiate the infected and non-infected cases. Ascitic fluid study is essential to identify infection. Culture of ascitic fluid is not always diagnostic of infection.

**Keywords:** Chronic liver disease, CLD, Bangladesh, Ascites, bacterial infection.

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## INTRODUCTION

Cirrhosis defined as a diffuse liver disease where fibrosis has resulted in a conversion of the liver architecture into structurally abnormal nodules (according to WHO)[1]. This distortion of liver architecture leads to compression of hepatic vascular & biliary structures, creating further imbalance in the delivery of nutrients, oxygen & metabolites. Even after the original insult has been controlled or stopped, the cirrhotic state persists. Chronic liver disease (CLD) is

not rare among paediatric population in Bangladesh. Karim *et al.* 1990 found that 4% of hospitalized children in the department of general paediatrics and paediatric gastroenterology & nutrition were due to liver disease and among them 40% had CLD[2]. CLD is a common medical problem encountered in our clinical practice causing much more morbidity & mortality. Patients with chronic liver disease are particularly susceptible to infections with a higher prevalence in cirrhotics [3]. Ascites is a frequent complication of cirrhosis. Ascites is pathologic fluid accumulation

within the abdominal cavity. The major factor of ascites formation is splanchnic vasodilation [4]. The peripheral arterial vasodilatation theory of ascites formation in chronic liver disease predicts that sodium and water retention in response to peripheral vasodilatation increases plasma volume enough to cause ascites formation. Spontaneous bacterial peritonitis is a frequent complication of ascites in children with chronic liver disease. Spontaneous bacterial peritonitis (SBP) is defined as infected ascites in absence of recognizable secondary cause of infection [5]. It is a frequent and severe complication of cirrhotic ascites, first described in the middle of the 1960s [6]. Three groups according to the long-accepted classification; Group I spontaneous bacterial peritonitis (SBP) in which the cell count was  $\geq 250/\text{mm}^3$  and culture was positive. Group II culture negative neutrocytic ascites (CNNA) with cells  $\geq 250/\text{mm}^3$  and culture negative. Group III negative cases in which cells  $\leq 250/\text{mm}^3$  and culture negative [7]. As mentioned above, SBP and CNNA are identical, both from the clinical point of view and the therapeutic approach. Its occurrence is related to low protein levels and impaired opsonic activity in ascitic fluid. Most episodes of spontaneous bacterial peritonitis are monomicrobial and produced by enteric bacteria. Of such episodes, 67% involve gram-negative bacteria, *Escherichia coli* being the most frequently isolated organism [8]. Bacteria participating in SBP come from the digestive tract. Extraintestinal bacteria such as those from the respiratory apparatus, urogenital tract or skin are much less frequent. Catheters and other equipment used during invasive procedures represent another possible source of infection. It is currently hypothesised that SBP follows an episode of bacteremia during which, due to the constant exchange of fluids between the peritoneal and intravascular space, ascitic fluid gets infected [9]. It can occur in 10-30% of cirrhotic patients with ascites, having an in-hospital mortality rate of around 30 to 50% [10,11]. The risk of SBP recurrence is around 70% per year [4]. There is around 10% probability of developing SBP in patients with the end stage liver disease and ascites over a period of one year [12]. Viera *et al.* found that the prevalence of infected ascites was 29.2% [13]. With the exception of serum albumin, there were no differences in the clinical & biochemical features of patients with infected and noninfected Ascites [13]. Mortada *et al.* found that culture of the ascitic fluid is not always diagnostic of infection [14]. Biochemical parameters of the ascitic fluid definitely add to the diagnostic accuracy. Ascites is also poor prognostic sign. Characteristically, it develops during late stages of the disease. Ascites is secondary to impaired humoral and cellular immune responses and here ascitic fluid acts as a culture medium for several bacterial agents [15]. Ascitic fluid bacterial infection of cirrhotic patients may be asymptomatic in 30% of cases [16]. Symptomatic ascitic fluid bacterial infection means patients who have fever, abdominal pain & tenderness either singly or in combination in patients of

liver cirrhosis. Asymptomatic ascitic fluid bacterial infection means patients who have none of these symptoms & signs [17]. A positive ascitic fluid culture for bacteria was considered essential to establish the diagnosis of SBP. However relying on ascitic fluid culture for diagnosis of SBP has the disadvantages of poor sensitivity & relatively long time before the results are known. To circumvent problem with culture, the ascitic fluid white blood cell (WBC) and PMN counts have become the standards for making a diagnosis of SBP [18]. So ascitic fluid study is helpful to confirm the diagnosis of ascitic fluid infection from both symptomatic & asymptomatic patients to prevent morbidity and mortality. Mortality rate decreases with earlier diagnosis & aggressive treatment with broad spectrum intravenous antibiotics. The recurrence rate for SBP is high, and oral antibiotic prophylaxis with either norfloxacin or ciprofloxacin has reduced recurrence [19].

Therefore the aim of this study is to see the variants of spontaneous ascitic fluid infection in children with chronic liver disease.

### Objective

To see the variants of ascitic fluid infection in children with chronic liver disease

## MATERIALS AND METHODS

It is a cross sectional observational study, conducted in department of Pediatric Gastroenterology & Nutrition, Bangabandhu Sheikh Mujib Medical University, Bangladesh. Duration of study was one & half year (January 2016 through July 2017). All the diagnosed cases of chronic liver disease with ascites admitted into the department of Pediatric Gastroenterology & Nutrition, Bangabandhu Sheikh Mujib Medical University during the study period were included in this study. Sample size was 30. Patients who received antibiotic within last seven days, having feature of encephalopathy & parents refused to give consent were excluded from this study.

### Operational definitions

#### Cirrhosis

Cirrhosis defined as a diffuse liver disease where fibrosis has resulted in a conversion of the liver architecture into structurally abnormal nodules [1].

#### Ascites

Ascites is pathologic fluid accumulation within the peritoneal cavity [20].

#### Variants of ascitic fluid infection [21]

I) Spontaneous bacterial peritonitis (SBP): Spontaneous bacterial peritonitis (SBP) means ascitic fluid neutrophil counts  $\geq 250/\text{mm}^3$  and positive culture.

II) Culture negative neutrocytic ascites (CNNA): Culture negative neutrocytic ascites (CNNA)

means ascitic fluid neutrophil counts  $\geq 250/\text{mm}^3$  but culture negative.

III) Monomicrobial non-neutrocytic bacterascites: Monomicrobial non-neutrocytic bacterascites means ascitic fluid neutrophil counts  $< 250/\text{mm}^3$  but culture positive.

IV) Polymicrobial bacterascites: Polymicrobial bacterascites means ascitic fluid neutrophil counts  $< 250/\text{mm}^3$  but multiple organisms' positive culture.

V) Secondary bacterial peritonitis: Secondary bacterial peritonitis means ascitic fluid neutrophil counts  $\geq 250/\text{mm}^3$  and multiple organisms' positive culture.

Infected group: Infected group means ascitic fluid neutrophil counts  $\geq 250/\text{mm}^3$  and/or culture positive (SBP or CNNA).

Non infected group: Non infected group means ascitic fluid neutrophil counts  $< 250/\text{mm}^3$  and/or culture positive.

## Procedures

Informed written consent taken from parents. Data were collected using a structured questionnaire (research instrument) containing all the variables of interest and from investigation reports. We had done complete blood counts, Serum ALT, AST, Bilirubin, Albumin, Alkaline phosphatase, GGT, PT, APTT, HBsAg(ELISA), Anti-HCV(ELISA), Serum ceruloplasmin, K-F ring, 24 hours urinary copper basal & after challenge, scintigraphy of hepatobiliary system, Anti-LKM-1 antibody, ANA, Anti Sm, Total IgG, tTG, LDH, Liver biopsy, USG & endoscopy for selected children. All invasive procedure was done in the department of Paediatric Gastroenterology & Nutrition, BSMMU. Blood collection and ascitic tap were done under aseptic measures. Paracentesis was done before antibiotic therapy or 7 days of stopping antibiotic in both symptomatic & asymptomatic children. 25 ml ascetic fluid was aspirated. 5ml of ascitic fluid was sent to hospital clinical pathology lab for cytology under microscopic examination & Gram staining. 5 ml ascitic fluid was sent to biochemistry department for total protein & LDH by auto analyzer. 15 ml ascitic fluid was inoculated in a automated blood culture system or conventional culture media containing tryptic soy broth at bed side. Tryptic soy broth media was facilitate the growth of aerobic microorganisms specially *Escherichia coli*, *Haemophilus influenzae*, *Neisseria meningitidis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus pneumonia*, *Streptococcus pyogenes group A*, *Alcaligenes faecalis* and antibiotic sensitivity were seen. Variants of ascitic fluid infection were identified on the form of by ascitic fluid neutrophil counts & culture report.

## Statistical methods

Statistical analysis was done using the Statistical Package for Social Science (SPSS, version 20). Descriptive statistics were used for demographic and baseline data and was presented as mean  $\pm$  standard deviation (SD), median (range), number or percentage. Chi-square test or Fisher's Exact test was used for categorical variable (age, sex, ascitic fluid color, culture positivity or negativity, etc.) while student *t*-test and Mann Whitney U test were used for comparison of continuous variable (ascitic fluid total protein, ascitic fluid LDH, ascitic fluid total WBC counts, ascitic fluid neutrophil counts, etc). Comparison was done between the infected & non-infected group. For all statistical test *p* value of less than 0.05 was considered as statistically significant.

## RESULTS

### Characteristics of the studied children

#### Demography of the studied children (n=30)

The demographic data of the total 30 children with chronic liver disease were included for this study.

#### Age distribution

It was observed that the age range of the children were from 6 months to 13 years and mean age was  $6.4 \pm 4.2$  years. The highest (43.3%, n=13) incidence of CLD was found in the age group of 5-10 years.

Sex distribution of the studied children a male predominance was observed in the study. Male were 60% (n=18) and female 40% (n=12).

#### Etiology of chronic liver disease found in studied children (n=30)

Wilson's disease (WD) was the commonest 9(30%) cause of the CLD in this study. Other causes were biliary cirrhosis 6(20%), hepatitis B virus 2 (6.7%), caroli disease 2(6.7%), lipid storage disease 2(6.7%), budd chiari syndrome 1(3.33%), hepatitis B virus with WD 1(3.33%) & cryptogenic 7 (23.3%).

#### Presenting symptoms of infected and non-infected children

Gradual abdominal distention was present in 30(100%) of both infected and non-infected children. History of jaundice was present in 18(72%) of non-infected and in 5(100%) of infected children. History of fever and abdominal pain were in all infected patients 5(100%) but in non-infected patients 13(52%) & 6(24%) respectively and these difference is significantly higher ( $p < 0.05$ ). History of passage of pale stool was present in 5(20%) of non-infected children and in 1(20%) of infected children. Vomiting was present in 7(28%) of non-infected and in 3(60%) of infected children. History of hematemesis and melena were present in 2(8%) of non-infected children & in 1(20%) of infected children (Table-I).

**Table-I: Presenting symptoms among infected and non-infected children**

Symptoms	Non infected group (n=25) N(%)	Infected group (n=5) N(%)	Total N (%)	p value
History of Gradual abdominal distension	25(100)	5(100)	30(100)	
History of Jaundice	18(72)	5(100)	23(76.67)	0.177 <sup>a</sup>
History of Fever	13(52)	5(100)	18(60)	0.046 <sup>a</sup>
History of Abdominal pain	6(24)	5(100)	11(36.67)	0.001 <sup>a</sup>
History of vomiting	7(28)	3(60)	10(33.33)	0.300 <sup>b</sup>
History of Passage of pale stool	5(20)	1(20)	6(20)	1.000 <sup>b</sup>
History of Haematomesis	2(8)	1(20)	3(10)	0.433 <sup>b</sup>
History of Malena	2(8)	1(20)	3(10)	0.433 <sup>b</sup>

<sup>a</sup> Chi-square test<sup>b</sup> Fisher's Exact Test**Difference of presenting signs of the infected and non-infected children (n=30)**

Ascites was present in 30(100%) of both non infected and infected children. Jaundice was present in 16(64%) of non- infected and in 5(100%) of infected children. Hepatomegaly was present in 14(56%) of non-infected children and in 3(60%) of infected children. Splenomegaly was present in 10(40%) of non-infected and in 3(60%) of infected children. Stigmata of CLD was seen in 9 (36%) of non-infected and in 2(40%) of

infected children. KF ring was seen in 4 (16%) of non-infected and in 1(20%) of infected children the difference of above value between infected & none infected group are not significant ( $p>0.05$ ). Body temperature  $>99^{\circ}\text{F}$  was more common in infected group 4(80%) than non-infected group 2(8%) and the difference of value is significant ( $p=0.003$ ). Abdominal tenderness was also more common in infected group 3(60%) than non-infected group 2(8%) and the difference of value is significant ( $p=0.022$ )(Table-II).

**Table-II: Presenting signs of the non-infected and infected children (n=30)**

Sign	Non infected group (n=25) N (%)	Infected group (n=5) N(%)	Total(n=30) N(%)	p value
Ascites	25(100)	5(100)	30(100)	
Jaundice	16(64)	5(100)	21(70)	0.109 <sup>a</sup>
Hepatomegaly	14(56)	3(60)	17(56.67)	1.000 <sup>b</sup>
Splenomegaly	10(40)	3(60)	13(43.33)	0.628 <sup>b</sup>
Stigmata ofCLD	9(36)	2(40)	11(36.67)	1.000 <sup>b</sup>
KF ring	4(16)	1(20)	5(16.67)	0.539 <sup>b</sup>
Temperature $>99^{\circ}\text{F}$	2(8)	4(80)	6(20)	0.003 <sup>b</sup>
Abdominal tenderness	2(8)	3(60)	5(16.67)	0.022 <sup>b</sup>

<sup>a</sup> Chi-square test<sup>b</sup> Fisher's Exact Test**Compare the ascitic fluid infection in symptomatic and asymptomatic children**

Total seven (23.33%) patients were symptomatic (ie.they had features of ascitic fluid infection like fever with abdominal pain and/or

tenderness) and 23 (76.67%) were asymptomatic. Symptomatic patients were more common in infected group 4(80%) than in non-infected group 3(12%) and this difference is statistically highly significant ( $p=0.006$ ) (Table-III).

**Table-III: Ascitic fluid bacterial infection in symptomatic and asymptomatic children (n=30)**

Character	Non infected(n=25) N (%)	Infected(n=5) N(%)	Total (n=30) N=%	p value
Symptomatic	3 (12)	4 (80)	7 (23.33)	0.006 <sup>b</sup>
Asymptomatic	22 (88)	1 (20)	23 (76.67)	

<sup>b</sup> Fisher's Exact Test**Laboratory findings of the studied children****Haematological profile of the children (n=30)****Total count of WBC in infected and non-infected children**

Total six (20%) children had blood total WBC count  $>11000/\text{mm}^3$ . Total WBC counts  $>11000/\text{mm}^3$  was in 2(40%) of infected children & in 4(16%) of non-

infected children which is statistically not significant ( $p=0.254$ ). (Table-IV)

**Blood neutrophil count in infected and non-infected children**

Total five (16.67%) children had blood neutrophil counts  $>70\%$ . Neutrophil counts  $>70\%$  were

in 1(20%) infected children & in 4(16%) non-infected children which is statistically not significant ( $p = 1.000$ )

(Table-IV).

**Table-IV: Total blood WBC and neutrophil count of the infected and non-infected children (n=30)**

Laboratory findings	Infected group (n=5) N(%)	Non infected Group (n=25) N(%)	Total (30) N(%)	p value
Total counts of blood WBC >11000/mm <sup>3</sup>	2(40)	4(16)	6(20)	0.254 <sup>b</sup>
Total counts of blood WBC <11000/mm <sup>3</sup>	3(60)	21(84)	24(80)	
Blood neutrophil count >70%	1(20)	4(16)	5(16.67)	1.000 <sup>b</sup>
Blood neutrophil counts <70%	4(80)	21(84)	25(83.33)	

<sup>b</sup> Fisher's Exact Test

#### Ascitic fluid characteristics of studied children

##### Cytological profile of the studied children (n=30)

Ascitic fluid neutrophil count  $\geq 250/\text{mm}^3$  was in total 5 (16.67%) patients. Median ascitic fluid total WBC count of infected and non-infected children were 1200/ mm<sup>3</sup> (range 600-10,000/mm<sup>3</sup>) and 100/ mm<sup>3</sup>

(range 20-600/mm<sup>3</sup>) respectively which is statistically highly significant ( $p = 0.001$ ). Median ascitic fluid absolute neutrophil count of infected and non-infected children were 720/mm<sup>3</sup> (range 360-3600/ mm<sup>3</sup>) and 30/mm<sup>3</sup> (range 3-192/ mm<sup>3</sup>) respectively which is also statistically highly significant ( $p = 0.001$ ) (Table-V).

**Table-V: Cytological profile of ascitic fluid (n=30)**

Laboratory findings of ascitic fluid	Infected group(n=5)	Non infected group(25)	p value
<sup>c</sup> Ascitic fluid WBC counts/ mm <sup>3</sup> (Range)	1200 (600-10,000)	100 (20-600)	0.001 <sup>b</sup>
<sup>c</sup> Ascitic fluid neutrophil counts/mm <sup>3</sup> (Range)	720 (360-3600)	30 (3-192)	0.001 <sup>b</sup>

<sup>a</sup> Independent t-test

<sup>b</sup> Mann Whitney U-test

#### Biochemical profile of the studied children (n=30)

Median ascitic fluid LDH of infected and non-infected children were 138 U/L (range 28-283 U/L) and 54 U/L (range 13-237 U/L) respectively which is statistically not significant ( $p=0.07$ ). The mean ascitic

fluid total protein (AFTP) was  $0.36 \pm 0.23$  gm/dl in infected group and  $1.28 \pm 1.13$  gm/dl in non-infected group which is statistically not significant ( $p = 0.087$ ). (Table-VI).

**Table-VI: Biochemical profile of ascitic fluid (n=30)**

Laboratory findings of ascitic fluid	Infected group(n=5)	Non infected group(25)	p value
<sup>c</sup> Ascitic fluid LDH (U/L) (Range)	138 (28-283)	54 (13-237)	0.07 <sup>b</sup>
Ascitic fluid total protein(gm/dl) mean $\pm$ SD	$0.36 \pm 0.23$	$1.28 \pm 1.13$	0.087 <sup>a</sup>

<sup>a</sup> Independent t-test

<sup>b</sup> Mann Whitney U-test

#### Ascitic fluid Gram stain

Gram stain positive were found in 13(43.33%) children. Ascitic fluid positive Gram stain was 2(40%)

in infected group and 11 (44 %) in non-infected group which is statistically not significant ( $p = 0.869$ ) (Table-VII).

**Table-VII: Ascitic fluid Gram stain among infected & non infected children (n=30)**

Ascitic fluid Gram stain	Infected group (n=5) N(%)	Non infected group (n=25) N(%)	Total (n=30) N(%)	P value
Positive Gram stain	2(40)	11(44)	13(43.33)	1.000 <sup>b</sup>
Negative Gram stain	3(60)	14(56)	17(56.67)	

<sup>b</sup> Fisher's Exact Test

### Ascitic fluid culture

Among 30 studied children none had culture positive ascitic fluid bacterial infection, so antibiotic sensitivity pattern of pathogenic organism could not be seen. Though 5 children (CNNA variants) had ascitic fluid bacterial infection evidenced by ascitic fluid neutrophil count of  $\geq 250$  cells/mm<sup>3</sup>. (Table-VIII).

### Variants of ascitic fluid bacterial infections

Variants of ascitic fluid infection was determined on the basis of ascitic fluid polymorphonuclear neutrophil count and ascitic fluid culture results. Table VIII shows the variants of ascitic fluid bacterial infection in this study. In the present study, 5 (16.67%) children had ascitic fluid bacterial infection which was culture negative neutrocytic ascites (CNNA). Other children had no ascitic fluid bacterial infection.

**Table -VIII: Different variants of ascitic fluid bacterial infection in studied children (n=30)**

Type of ascitic fluid bacterial infections	Present Number	%
Spontaneous bacterial peritonitis	0	00
Culture-Negative Neutrocytic Ascites (CNNA)	5	16.67
Secondary Bacterial Peritonitis	0	00
Monomicrobial Non-neutrocytic (MNB) Bacterascites	0	00
Polymicrobial Bacterascites	0	00

## DISCUSSION

Present study was conducted at the department of Paediatric Gastroenterology and Nutrition, BSMMU to observe the variant of ascitic fluid bacterial infections in children with chronic liver disease (CLD). Thirty consecutive cases who met inclusion criteria of CLD were enrolled in the study. Demographic, clinical and biochemical features of studied subjects were analyzed.

Ascitic fluid polymorphonuclear cells increase with peritoneal infection [22]. In the present study, among the thirty studied children 5 children had ascitic fluid neutrophil count of  $\geq 250$  cells/mm<sup>3</sup> which is a diagnostic parameter of ascitic fluid bacterial infection.

The upper limit of the absolute PMN count in uncomplicated cirrhotic ascitic fluid is usually stated to be  $< 250$ /mm<sup>3</sup> [23]. For the diagnosis of different variants of ascitic fluid infection, ascitic fluid absolute neutrophil count is very important. Ascitic fluid absolute neutrophil count in spontaneous bacterial peritonitis (SBP), culture negative neutrocytic ascites (CNNA) and secondary bacterial peritonitis is  $\geq 250$ /mm<sup>3</sup>, but ascitic fluid absolute neutrophil count is  $< 250$  / mm<sup>3</sup> in monomicrobial nonneutrocytic bacterascites (MNB) and polymicrobial bacterascites[24]. In the present study, median ascitic fluid neutrophil count was 720/mm<sup>3</sup>, with a range of 360-3600/mm<sup>3</sup> among infected (CNNA variants) children and median ascitic fluid neutrophil count was 30/mm<sup>3</sup> with a range of 3-192/mm<sup>3</sup> among non-infected children which is statistically significant (p value=0.001). Naglaa *et al.* found that median ascitic fluid absolute neutrophil count was 700/mm<sup>3</sup>, with a range of 400-1800/ mm<sup>3</sup> among infected (SBP) and 100/mm<sup>3</sup> with a range of 50-150/ mm<sup>3</sup> among non-infected patient [25]. So, the findings of the present study are similar to findings of the study done by Naglaa *et al.*[25].

In this study, most of the children were  $< 10$  years of age and the highest incidence of CLD with ascites was found in the age group of 5-10 years. The age range of the studied children was 6 months to 13 years and the mean age was  $6.4 \pm 4.2$  years. Mortada *et al.* showed that patient's age ranged from 6 months to 11 years, with a mean age  $5.1 \pm 3.3$  years which nearly similar with present study [14].

In this study, male were 18 (60%) and female were 12 (40%). This male predominance 48 (59.26%) was also observed in the study done by Sayed *et al.*[5]. Fever, abdominal pain and tenderness are features of ascitic fluid infection. These features, however, may be absent or subtle [22]. In the present study, among 30 children, 7 (23.33%) children were symptomatic (they had features of ascitic fluid bacterial infection like fever, abdominal pain and/or tenderness) and 23 (76.67%) were asymptomatic. Among total 7 symptomatic children 4(80%) were infected and 3 (12%) were non-infected. Among total 23 asymptomatic children 1 (20%) was infected and 22 (88%) were none infected, and this difference is statistically significant (p=0.006). The reason of presence of features of ascitic fluid infection in non-infected children may be presence of infection other than ascitic fluid infection like UTI or pneumonia etc. The reason of absence of symptoms of infection among the infected children may be due to immuno suppression. Fernandez *et al.* in a study, showed that the most common infections in cirrhosis were SBP (25%), followed by UTI (20%) and pneumonia (15%)[9]. So, the causes of symptoms of ascitic fluid infection (i.e. fever, abdominal pain) in ascitic fluid non-infected children in the present study may be due to other infection like UTI. In another study done by Sayed *et al.* (2007) it was found that 65% were symptomatic, 35% were asymptomatic among infected group & 22.95% symptomatic, 77.05% asymptomatic among non-infected group (p=0.001)[5]. Mortada *et al.* showed

that about 92.3% patients were fever among infected group & 53% among non-infected group ( $p < 0.05$ ) that similar with present study [14]. Safia *et al.* found that fever, abdominal pain & tenderness are not significantly different between SBP & non SBP group ( $p > 0.05$ ) [26].

Systemic leukocytosis is noted in ascitic fluid infection. In the present study, total count of blood WBC ranged from 3,500-19,000/mm<sup>3</sup>. A total 6 children had WBC count of  $> 11,000/\text{mm}^3$  of which 2(40%) were infected and 4(16%) were non-infected which is statistically not significant ( $p = > 0.05$ ). The rise of total WBC count in non-infected children may be due to the other infections like UTI, pneumonia etc. Immuno suppression may be the cause of this low WBC count among the infected children. Safia *et al.* shows mean total WBC counts 12070 $\pm$ 6590/mm<sup>3</sup> among infected group & total WBC counts 9360 $\pm$ 9710/mm<sup>3</sup> among non-infected group ( $p = 0.185$ ) [26]. But Nepal *et al.* found that blood total WBC counts & neutrophil percentage were significantly higher in SBP group than non SBP group ( $p < 0.05$ ) [27].

In sterile ascites, ascitic fluid white blood cell count is usually less than 100/mm<sup>3</sup> with a predominance of mononuclear cells and a low number of polymorphonuclear cells [22]. In the present study, median ascitic fluid WBC counts among infected (CNNA) children was 1200/mm<sup>3</sup> with a range of 600-10,000/mm<sup>3</sup> and among non-infected children it was 100/mm<sup>3</sup> with a range of 20-600/mm<sup>3</sup> which is statistically significant ( $p = 0.001$ ). Sayed *et al.* showed that mean ascitic fluid WBC count were 3363.1 $\pm$ 6209/mm<sup>3</sup> and 96.87 $\pm$ 91.23/mm<sup>3</sup> in infected & non infected children respectively ( $p = 0.000$ ) [5]. Difference of ascitic fluid WBC count among infected patients and non-infected group is due to inflammatory reactions. Safia *et al.* found that mean ascitic fluid total WBC counts were 1207 $\pm$ 6.59 & 936 $\pm$ 971 among SBP & non SBP group which statistically not significant ( $p = 0.185$ ) [26].

Patients with ascitic fluid total protein of  $< 1\text{gm/dl}$  are the most prone to develop ascitic fluid infection [28]. The bactericidal capacity of the ascitic fluid is proportional to the ascitic fluid protein concentration [29]. In this study mean ascitic fluid total protein was 0.36 $\pm$ 0.23gm/dl and 1.28 $\pm$ 1.13gm/dl among infected (CNNA) and non-infected children respectively ( $p = 0.087$ ). Syed *et al.*, (2007) showed 81 patient with CLD with ascites had mean ascitic fluid total protein was 1.1 $\pm$  0.72 g/dl among infected and 1.2 $\pm$ 0.75 g/dl among non-infected patients ( $p = 0.61$ ) [5]. Higher mean ascitic fluid protein in non-infected group than infected group was found in a study conducted by Asmaa *et al.* ( $p = 0.001$ ) which contrast to my study [30].

In the present study, ascitic fluid Gram stain positive was in 13(43.33%) children. Ascitic fluid positive Gram stain was 2(40%) in infected group and

11 (44 %) in non-infected group which is statistically not significant ( $p = 1.000$ ). This high percentage positive Gram stain but negative culture may be due to ascitic fluid contamination from skin when collect or during slide preparation. In contrast to this study, Surendra, found that positive Gram stain in SBP is only 7% & 10% in uncentrifused & centrifused sample respectively [31].

For the purpose of diagnosis and classification of ascitic fluid infection, culture of the ascitic fluid is essential. Ascites bacteriological culture is negative in approximately 40% of adult patients with clinical manifestations suggestive of SBP [19]. In fact, the sensitivity of culture in detecting bacterial growth in neutrocytic ascites (i.e., ascitic fluid with a PMN counts  $\geq 250$  cells/mm<sup>3</sup>) varies widely depending on the method of culture used. In published studies, the conventional method of culture has been found to detect bacterial growth in approximately 50 % of neutrocytic samples [24]. In the present study in children, out of a total 30 children, ascitic fluid culture result was found negative in all children, though, 5(16.67%) children had neutrocytic ascites (PMN count  $\geq 250/\text{mm}^3$ ). Similar study in our department of CLD with ascites done by Liaquat, showed that out of a total 35 patients ascitic fluid culture in conventional method showed no growth of organism, though in his study, out of a total 35 patients, 16 (45.7%) had PMN count of  $\geq 250/\text{mm}^3$  [32]. Sarker *et al.* showed that no growth of organism but 8 (22.8%) had PMN count of  $\geq 250/\text{mm}^3$  [21]. Gene probes are now commercially available for the detection of bacteremia, hopefully, it will lead to rapid (30 minute) and accurate detection of organisms in ascitic fluid [33]. Sideris *et al.* in a study showed that ascitic fluid culture positive in only 2.28 cases [34]. Ascitic fluid culture was found negative in the present study; the reason may be due to i) the media used was not enriched enough, ii) paucity of bacteria in ascitic fluid, iii) anaerobic organisms are not identified by conventional culture media, iv) selected aerobic organisms are identified by selected media, v) neutrophil mediated killing of bacteria. On the basis of present facility only aerobic culture was done, though anaerobes can cause ascitic fluid infection rarely (1%). The infrequency of anaerobic SBP is due to the the relatively high PO<sub>2</sub> of ascites and the inability of anaerobes to translocate across the gut mucosa [35]. Most episodes of CNNA are diagnosed using insensible cultured methods where there are insufficient numbers of bacteria to reach the threshold of detectability [36]. The conventional method of culture probably requires at least 100 organisms/ml [23]. However even when optimal culture methods are used a small percentage of patients grow no bacteria in their neutrocytic ascitic fluid [37].

In another study it was reported that CNNA was more common than SBP. Andrew Sideris *et al.* showed in their study that a total of 219 patients were

admitted with ascites due to cirrhosis during the study period. Of them a total of 15 (6.85%) patients had ascitic fluid infection. Out of 15 patients with ascitic fluid infection, only 13.33% had spontaneous bacterial peritonitis while 86.67% had CNNA type[34]. In the present study all the children (16.67%) of ascitic fluid infection were CNNA type.

In a prospective study it was shown that 32-34% of cirrhotic patients developed bacterial infection either at the time of admission or later during their hospital stay.<sup>38</sup> In the present study culture negative neutrocytic ascites (CNNA) was the only variety of ascitic fluid infection. Infected cases may be asymptomatic. Clinical features of ascitic fluid infection are needed to differentiate the infected and non-infected cases. Ascitic fluid study is essential to identify infection. Culture of ascitic fluid is not always diagnostic of infection.

## CONCLUSION

Culture negative neutrocytic ascites (CNNA) was the only variety of ascitic fluid infection in this study. Polymorphonuclear neutrophil cell count in ascitic fluid was found significantly higher in this group of children. Infected cases may be asymptomatic. Clinical features of ascitic fluid infection are needed to differentiate the infected and non-infected cases. High degree of suspicion is essential to diagnose ascitic fluid infection. Ascitic fluid study is also essential to identify infection. Culture of ascitic fluid is not always diagnostic of infection. Further studies with larger sample size are necessary to know the actual fact about bacterial infection of ascitic fluid in children with chronic liver disease.

## Limitations of the study

- Time and resources were limited.
- Sample size was small.
- Only aerobic culture was done.
- This was a hospital based single centre study.

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